IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT: Curiel *et al.*	§	**ART UNIT: 1632**
	§	
FILED: September 9, 1999	§	
	§	
SERIAL NO.: 09/393,173	§	**EXAMINER:**
	§	**Wehbe, A.M.S.**
FOR: Adenoviral Vector Encoding Pro-Apoptotic Bax Gene and Uses Thereof	§	
	§	
	§	**DOCKET: D6163**

Assistant Commissioner for Patents
BOX AF
Washington, DC 20231

DECLARATION UNDER 37 C.F.R. § 1.132

Dear Sir:

I, David T. Curiel, do hereby state as follows:

I am a co-inventor of the above-referenced patent application. I have read U.S. patent application serial no. 09/393,173 and I am aware of the content of the Office Action, including all prior art cited against the '173 application.

An issue relating to the patentability of the claimed methods is the degree of enablement provided by Applicants' specification. The following data are presented as evidence of enablement commensurate with the scope of the claims:

To demonstrate the capacity of adenovirus to deliver *bax* *in situ* and to sensitize previously irradiated tumor, an *in vivo* therapeutic experiments were performed. Nude mice (n=5/group) bearing established subcutaneous human glioblastoma cells D54MG received either radiation alone, irrelevant virus (Ad/Bax+Ad/Luc) with radiation, or Ad/Bax+Ad/Cre with or without radiation. Radiation (5 Gy) and viruses were administered every other day for four times.

Animals treated with Ad/Bax+Ad/Cre without radiation, as well as those non-treated, showed rapid tumor growth, and had to be sacrificed after 5 weeks (Fig. 1). Animals treated with radiation alone or radiation with irrelevant viruses showed a transient inhibition of their growth although the tumor grew back aggressively, and the animals had to be sacrificed after 8-10 weeks. In contrast, animals treated with Ad/Bax+Ad/Cre and radiation showed significant regression and inhibition of tumor growth over a 6-month time period. Of these mice, 60% had no evidence whatsoever of tumor. Thus, combined treatment of *bax* and radiation is uniquely able to completely eradicate malignant glioma tumor nodules in this mice model.

To show that Bax induces apoptosis in normal human astrocytes, and to demonstrate that it has sensitizing effects to radiation, astrocytes were exposed to 0 or 8 Gy radiation and infected with 100 PFU of Ad/Bax+Ad/Cre or irrelevant viruses (Ad/CD+Ad/Cre). Six days later, apoptosis was determined. Although Bax induced some apoptosis over that observed in uninfected controls (24% vs. 10% respectively), the levels were only slightly higher than those induced by irrelevant control viruses (18%) (Fig. 2A). Most importantly, the addition of radiation provoked a minor and insignificant ($p=0.267$) increase in apoptosis. This effect was small compared to the much greater increase of apoptosis observed with radiation in all the glioblastoma cell lines examined. To confirm this result, a cell proliferation assay was performed. Astrocytes treated with Ad/Bax and Ad/Cre or irrelevant virus showed no significant inhibition of cell proliferation (Fig. 2B). In addition, cell proliferation of irradiated cells did not differ significantly with or without viral treatment. Thus, Bax does not appear to sensitize normal astrocytes to the effect of radiation.

In conclusion, these data demonstrate a synergistic radiosensitizing effect of Bax in refractory glioblastoma cell lines after gene delivery

via recombinant adenovirus. This result was confirmed in an *in vivo* murine xenograft model of glioblastoma. Toxicity was selectively induced in tumors, but normal astrocytes were spared. Thus, the combination of *bax* gene delivery and radiotherapy might have clinical utility for the treatment of malignant brain tumors. Accordingly, I respectfully submit that the scope of the claims 2-3 and 5-10 in the '173 application has a reasonable correlation to the scope of the enablement provided.

Figure Legends

Figure 1: Subcutaneous nodules of glioma were radiosensitized by intratumoral delivery of Bax. In this experiment, nude mice 4 to 6 week old were used. Animals were classified in 5 groups (n=5) according to treatment as follows: radiation alone, irrelevant virus with or without radiation (5 Gy x 4), and Ad/Bax+Ad/Cre with or without radiation (5 Gy x 4). Animals were injected subcutaneously into lower flanks with 2 x 10^7 D54MG cells, and then followed for nodule formation for 3 weeks. When nodules reached suitable size, the tumors were irradiated and viruses were injected intratumorally at an MOI of $1x10^9$ following each

administration of radiation. Nodules were then monitored up to 6 months for tumor size. Representative data from one of two similar experiments is shown.

Figure 2:Bax does not sensitize normal human astrocytes to radiation. Human astrocytes (2 x 10^5/well) were plated into 6-well plates, and irradiated with 8 Gy of ^{60}Co irradiation or mock irradiated 5 days later. Apoptosis was determined 6 days after irradiation (Fig. 2A). Results from a cell proliferation assay was shown in Fig. 2B.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or patent issued thereon.

Date: 1/30/03

Dr. David T. Curiel